SANTA CRUZ BIOTECHNOLOGY, INC.

Clathrin HC siRNA (m): sc-35066

BACKGROUND

Clathrin is a major cytosolic coat protein in pits and vesicles originating from the plasma membrane and the *trans*-Golgi network. In receptor-mediated endocytosis, receptor proteins are englufed by clathrin-coated vesicles. Clathrin is composed of three heavy chains and three light chains which associate non-covalently to form a triskelion structure. Clathrin heavy chain (HC) is composed of a terminal globular domain, a distal segment and a proximal segment containing a light chain binding site. The proximal segment of the Clathrin HC protein is essential for interactions between clathrin heavy chains and light chains which result in the formation of the triskelion structure.

REFERENCES

1. Pearse, B.M. 1987. Clathrin and coated vesicles. EMBO J. 6: 2507-2512.
2. Pearse, B.M. and Crowther, R.A. 1987. Structure and assembly of coated vesicles. Annu. Rev. Biophys. Biochem. 16: 49-68.
3. Kirchhausen, T., et al. 1987. Clathrin heavy chain: molecular cloning and complete primary structure. Proc. Natl. Acad. Sci. USA 84: 8805-8809.
4. Jackson, A.P. and Parham, P. 1988. Structure of human clathrin light chains. Conservation of light chain polymorphism in three mammalian species. J. Biol. Chem. 263: 16688-16695.
5. Liu, S.H., et al. 1995. Regulation of clathrin assembly and trimerization defined using recombinant triskelion hubs. Cell 83: 257-267.
6. Hunziker, W. and Geuze, H.J. 1996. Intracellular trafficking of lysosomal membrane proteins. Bioessays 18: 379-389.

CHROMOSOMAL LOCATION

Genetic locus: Cltc (mouse) mapping to 11 C.

PRODUCT

Clathrin HC siRNA (m) is a pool of 3 target-specific 19-25 nt siRNAs designed to knock down gene expression. Each vial contains 3.3 nmol of lyophilized siRNA, sufficient for a 10 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see Clathrin HC shRNA Plasmid (m): sc-35066-SH and Clathrin HC shRNA (m) Lentiviral Particles: sc-35066-V as alternate gene silencing products.

For independent verification of Clathrin HC (m) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-35066A, sc-35066B and sc-35066C.

STORAGE AND RESUSPENSION

Store lyophilized siRNA duplex at -20° C with desiccant. Stable for at least one year from the date of shipment. Once resuspended, store at -20° C, avoid contact with RNAses and repeated freeze thaw cycles.

Resuspend lyophilized siRNA duplex in 330 µl of the RNAse-free water provided. Resuspension of the siRNA duplex in 330 µl of RNAse-free water makes a 10 µM solution in a 10 µM Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

APPLICATIONS

Clathrin HC siRNA (m) is recommended for the inhibition of Clathrin HC expression in mouse cells.

SUPPORT REAGENTS

For optimal siRNA transfection efficiency, Santa Cruz Biotechnology's siRNA Transfection Reagent: sc-29528 (0.3 ml), siRNA Transfection Medium: sc-36868 (20 ml) and siRNA Dilution Buffer: sc-29527 (1.5 ml) are recommended. Control siRNAs or Fluorescein Conjugated Control siRNAs are available as 10 µM in 66 µl. Each contain a scrambled sequence that will not lead to the specific degradation of any known cellular mRNA. Fluorescein Conjugated Control siRNAs include: sc-36869, sc-44239, sc-44240 and sc-44241. Control siRNAs include: sc-37007, sc-44230, sc-44231, sc-44232, sc-44233, sc-44234, sc-44235, sc-44236, sc-44237 and sc-44238.

GENE EXPRESSION MONITORING

Clathrin HC (TD.1): sc-12734 is recommended as a control antibody for monitoring of Clathrin HC gene expression knockdown by Western Blotting (starting dilution 1:200, dilution range 1:100-1:1000) or immunofluorescence (starting dilution 1:50, dilution range 1:50-1:500).

To ensure optimal results, the following support reagents are recommended: 1) Western Blotting: use m-IgGκ BP-HRP: sc-516102 or m-IgGκ BP-HRP (Cruz Marker): sc-516102-CM (dilution range: 1:1000-1:10000), Cruz Marker™ Molecular Weight Standards: sc-2035, UltraCruz® Blocking Reagent: sc-516214 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use m-IgGκ BP-FITC: sc-516140 or m-IgGκ BP-PE: sc-516141 (dilution range: 1:50-1:200) with UltraCruz® Mounting Medium: sc-24941 or UltraCruz® Hard-set Mounting Medium: sc-359850.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor Clathrin HC gene expression knockdown using RT-PCR Primer: Clathrin HC (m)-PR: sc-35066-PR (20 µl, 596 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Lee, J.L., et al. 2014. Ultrasound enhanced PEI-mediated gene delivery through increasing the intracellular calcium level and PKC-δ protein expression. Pharm. Res. 31: 2354-2366.
2. Han, S.C., et al. 2016. Productive entry of foot-and-mouth disease virus via macropinocytosis independent of phosphatidylinositol 3-kinase. Sci. Rep. 6: 19294.

RESEARCH USE

For research use only, not for use in diagnostic procedures.

PROTOCOLS

See our web site at www.scbt.com for detailed protocols and support products.